

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

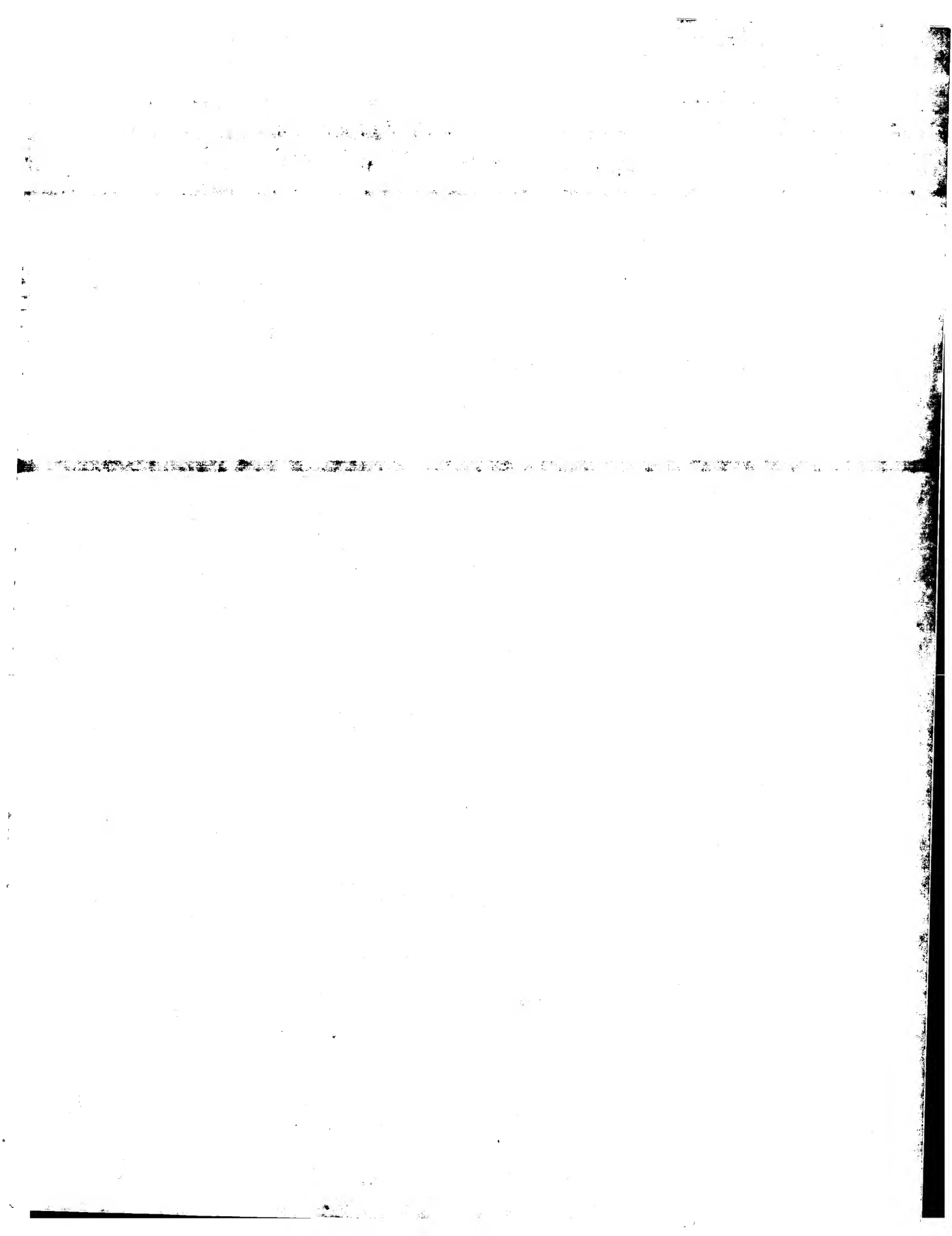
Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**



Brief Definitive Report

## Selective Reduction of T Cells Bearing Invariant V $\alpha$ 24J $\alpha$ Q Antigen Receptor in Patients with Systemic Sclerosis

By Takayuki Sumida,\* Akemi Sakamoto,\* Hideyuki Murata,\*  
 Yasuhiko Makino,\* Hidenori Takahashi,† Shoji Yoshida,\*  
 Kusuki Nishioka,‡ Itsuo Iwamoto,\* and Masaru Taniguchi†

From \*Second Department of Internal Medicine, †Center for Biomedical Science, School of Medicine, Chiba University, Chiba, Japan 260; ‡Department of Internal Medicine, Asahi General Hospital, Chiba, Japan 289-25; and †Division of Rheumatology and Molecular Immunology, Institute of Medical Science, St. Marianna University, School of Medicine, Kawasaki, Japan 216

**Summary**

A novel subset of T cells characterized by the expression of an invariant T cell antigen receptor (TCR) encoded by V $\alpha$ 24J $\alpha$ Q gene segments was investigated in patients with systemic sclerosis (SSc). Polymerase chain reaction analysis demonstrated that the V $\alpha$ 24 TCR repertoire was selectively used in CD4<sup>+</sup>CD8<sup>+</sup> double-negative T cells both in patients and in healthy individuals, while almost all families of TCRV $\alpha$  were expressed in single-positive T cell fractions. The V $\alpha$ 24<sup>+</sup> double-negative T cells were increased by approximately fivefold in patients. However, sequence analysis clearly showed significant differences in the V $\alpha$ 24 TCR repertoire dominating in patients and healthy donors. In healthy individuals, the invariant V $\alpha$ 24J $\alpha$ Q was expanded and comprised 20–50% of the total TCR- $\alpha$ , while their selective reduction was observed in SSc patients who also showed expansion of invariant V $\alpha$ 24 TCR other than V $\alpha$ 24J $\alpha$ Q. Analogous to murine invariant V $\alpha$ 14J $\alpha$ 281 TCR, these results suggest that T cells with invariant V $\alpha$ 24J $\alpha$ Q TCR would function as regulatory T cells, whereas T cells bearing other invariant V $\alpha$ 24 TCR in SSc patients could be autoaggressive T cells in nature.

**A**ccumulative evidence demonstrates that there are novel T cell subsets in the thymus characterized by their expression of cell-surface phenotypes of NK1.1 and TCR (1–4). This population belongs to either the double-negative (DN) CD4<sup>+</sup>CD8<sup>+</sup> or single-positive (SP) CD4<sup>+</sup>CD8<sup>+</sup> thymocytes but is apparently distinct from conventional T cells and NK cells having the germ line configuration of TCR genes. Recently, NK T cells have been found to be composed of a relatively large fraction of peripheral T cells comprising 5% of splenic T cells and 40% of bone marrow T cells (4). The most characteristic feature of NK T cells is that the majority bear an invariant TCR encoded by V $\alpha$ 14 and J $\alpha$ 281 gene segments with a one-base N region (4, 5). Thus, invariant V $\alpha$ 14 TCR is a marker for NK T cells. Moreover, NK T cells express a limited TCR V $\beta$  repertoire, including V $\beta$ 8, V $\beta$ 7, and V $\beta$ 2 (5).

Recent studies by Porcelli et al. (6) and Dellabona et al. (7, 8) indicate that the invariant V $\alpha$ 24J $\alpha$ Q sequence is preferentially expressed on DN  $\alpha/\beta$  T cells from healthy individuals. The homology of the nucleotide sequences is found to be 75% in the V $\alpha$ 24 and 90% in the CDR3 regions compared with murine V $\alpha$ 24 TCR (9). Therefore, the human V $\alpha$ 24 sequence is a homologue of murine V $\alpha$ 14 TCR. Another striking similarity to murine invari-

ant V $\alpha$ 14 T cells is that human peripheral DN T cells also express a limited TCR- $\beta$  repertoire including V $\beta$ 2, V $\beta$ 8, V $\beta$ 11, and V $\beta$ 13 (6–8, 10). Interestingly, the decrease in invariant V $\alpha$ 14J $\alpha$ 281 TCR expression in autoimmune prone mice correlates with disease development. It is thus likely that V $\alpha$ 14<sup>+</sup> NK T cells play a role in the regulation of autoimmune disease development.

Analogous to murine invariant V $\alpha$ 14 T cells, we investigated invariant V $\alpha$ 24J $\alpha$ Q TCR expression in patients with autoimmune diseases, such as systemic sclerosis (SSc). Surprisingly, invariant V $\alpha$ 24<sup>+</sup> TCR other than V $\alpha$ 24J $\alpha$ Q were dominant in SSc patients. However, invariant V $\alpha$ 24J $\alpha$ Q TCR were undetectable in patients, while 20–50% of TCR expressed in healthy individuals were invariant V $\alpha$ 24J $\alpha$ Q TCR. The results indicate that T cells bearing invariant V $\alpha$ 24J $\alpha$ Q function as regulatory T cells, while T cells with other invariant V $\alpha$ 24 TCR are likely to be autoaggressive in patients.

**Materials and Methods**

**Study Subjects.** Four patients diagnosed with SSc (11) were evaluated during the swelling phase of SSc. Three disease-free subjects were also examined as controls. All patients and healthy

subjects were of Japanese ancestry and were recruited from the Chiba University Hospital.

**Flow Cytometry.** PBL ( $1 \times 10^7$ ) from 20 ml of peripheral blood were isolated by Ficoll-Paque separation (Pharmacia Biotech Inc., Piscataway, NJ) and incubated with PE-coupled anti-CD4 (Leu-3a) plus anti-CD8 (Leu-2a) mAbs (Becton Dickinson & Co., Mountain View, CA) and FITC-conjugated mAb to  $\alpha/\beta$  TCR (mAb WT-31). The cells were analyzed by FACScan<sup>®</sup> with a logarithmic amplifier (Becton Dickinson & Co.).

**Purification of DN  $\alpha/\beta$  T Cells from PBL of SSc Patients and Healthy Subjects.** DN and SP  $\alpha/\beta$  T cells were sorted by FACStar<sup>®</sup> (Becton Dickinson & Co.) using PE-anti-CD4 plus anti-CD8 mAbs. The yields of DN and SP T cells were  $10^5$  and  $10^6$ , respectively. The purity of fractionated DN samples was confirmed by PCR with CD4 or CD8 primers (see Fig. 1 C).

**Preparation of RNA and PCR.** Total RNA (0.1–10  $\mu$ g) was prepared using Isogen (Nippon Gene Co. Ltd., Tokyo, Japan) from sorted DN  $\alpha/\beta$  T or SP  $\alpha/\beta$  T cells. cDNA synthesis and PCR were described elsewhere (12). Briefly, first-strand cDNAs were synthesized with oligo(dT) primer using 0.1–1  $\mu$ g of total RNA. PCR was performed with 21 different Va and Ca primers at 95°C for 1.5 min for denaturation, 62°C for 1.0 min for annealing, and 72°C for 1.0 min for extension, for 30 cycles on a DNA thermal cycler (Perkin-Elmer Corp., Norwalk, CT). The PCR products were hybridized with a <sup>32</sup>P-labeled Ca probe of 155 bp in length (13). The sequences of the Va and Ca primers were published previously (6, 14).

For confirmation of the purity of enriched samples, the cDNA from sorted DN  $\alpha/\beta$  T cells was amplified by PCR with CD4 and CD8a primers (15), and hybridized with the <sup>32</sup>P-labeled EcoRI fragment of the human CD4 gene (16) or the PstI/HincII fragment of the human CD8a gene (17). The CD4 or CD8 cDNA (1 ng) was used as a positive control.

**Quantitation of Va24<sup>+</sup> DN T Cells from SSc Patients and Healthy Subjects.** The relative amounts of Va24<sup>+</sup> DN T cells in PBL were measured by quantitative PCR. RNA was prepared from the sorted DN population derived from  $10^7$  of PBL T cells from three SSc patients and four healthy subjects. cDNAs ( $10^{-6}$  diluted) were used for PCR with primers for Va24 and Ca. For the standardization curve, Va24<sup>+</sup> cDNAs were serially diluted (corresponding to 0.01–10 pg DNA) and subjected to PCR with Va24 and Ca primers. PCR products were hybridized with a <sup>32</sup>P-labeled Ca probe, and the intensities of the bands were quantitated by an automated densitometer (Fujix BAS2000; Fujifilm I & I Co., Ltd., Tokyo).

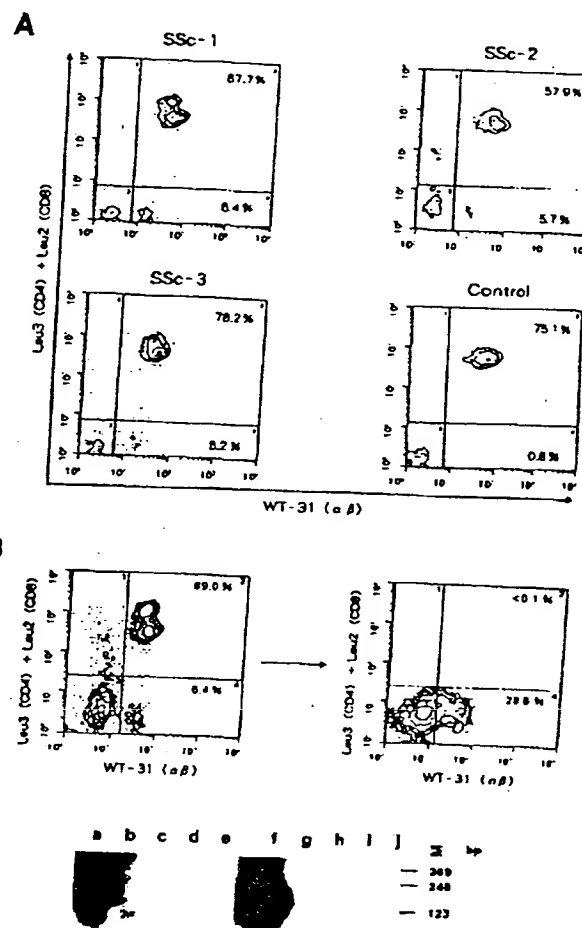
**Cloning and Sequencing of cDNAs Encoding TCR Va Genes.** Va24<sup>+</sup> cDNAs from DN  $\alpha/\beta$  T cells were amplified by primers with an EcoRI restriction site for Va24 (5'-CGAATTCCT-CAGCGATTGAGCCTCTAC-3') or Ca (5'-CGAATTCG-GTGAATAGGCAGACAGACTT-3'). DNA fragments with the expected size after digestion of PCR products with EcoRI were ligated to M13mp19 plasmids and sequenced by the dyc primer method.

**Plaque Hybridization.** The Va24<sup>+</sup> TCR cDNA libraries were generated by PCR using RNA from the DN population with primers for the Va24 and Ca. Recombinant plaques were transferred from DYT plates to a nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany) and were hybridized either with a Va24 probe (5'-CTCAGCGATTGAGCCTCTAC-3') or a 53-bp JaQ probe (5'-CAACCCTGGGGAGGCTATAC-3' for 5'-JaQ and 5'-AGGCCAGACAGTCAACTGAG-3' for 3'-JaQ).

**Statistical Analysis.** The statistical significance of the results was determined using the X<sup>2</sup>-test.

## Results and Discussion

**Predominant Expansion of TCR Va24<sup>+</sup> T Cells.** FACS<sup>®</sup> analysis of PBL from three patients with swelling-phase SSc clearly showed that their DN  $\alpha/\beta$  T but not DN  $\gamma/\delta$  T cell populations were increased in actual cell number ( $101/\text{mm}^3$ ,  $120/\text{mm}^3$ , and  $109/\text{mm}^3$ ) compared with healthy individuals (average  $28/\text{mm}^3$ ) (Fig. 1 A and Table 1). The in-



**Figure 1.** FACS<sup>®</sup> profiles and purification of DN  $\alpha/\beta$  T cells in PBL from SSc patients and healthy subjects. (A) DN  $\alpha/\beta$  T cells in PBL from three SSc patients (SSc-1, -2, and -3) and a healthy subject (control) were analyzed by FACS<sup>®</sup>, using PE-anti-CD4 plus anti-CD8 mAb and with FITC-WT-31 mAb (6.35, 5.69, 5.17, and 0.8%, respectively). (B) PBLs from an SSc patient were stained with PE-anti-CD4 plus anti-CD8 mAb and with FITC-WT-31 mAb (left panel). The DN population (right bottom panel) and CD4 and/or CD8 positive T cells (data not shown) were separated by FACStar<sup>®</sup>. DN  $\alpha/\beta$  T cells were enriched from 6.4 to 28.6%. (C) The purity of the fractionated DN populations was examined by reverse transcription-PCR with CD4 or CD8 primers followed by Southern blot analysis. Lane a, CD4 clone for positive control; lanes b and g, SSc-1; lanes c and h, SSc-2; lanes d and i, SSc-3; lanes e and j, healthy individual; lane f, CD8 clone for positive control. DNA size markers are shown to the right (in base pairs).

Table 1. Frequencies of Invariant Va24JaQ TCR Expression in Peripheral DN T

Source	Va24JaQ/ total Va24*		Cell number mm <sup>-3</sup>	
			DN $\alpha/\beta$ T	Va24JaQ T
SScDN-1	1/284 <sup>†</sup>	(0.4%)	101	0.4
SScDN-2	0/431 <sup>†</sup>	(0.0%)	120	0
SScDN-3	0/165 <sup>†</sup>	(0.0%)	109	0
Control-1	189/379	(49.9%)	25	12.5
Control-2	31/151	(20.5%)	35	7.0
Control-3	112/420	(26.7%)	26	6.9

\*Complementary DNA libraries generated by PCR with primers specific for Va24 and Ca were blotted on two separate filters and independently hybridized with the Va24-specific oligonucleotide probe and the JaQ probe, respectively. The ratio of invariant Va24JaQ/total Va24 was calculated by the number of positive plaques.

<sup>†</sup>Actual cell number of DN T cells bearing invariant Va24JaQ TCR was calculated on the basis of number of DN  $\alpha/\beta$  T cells, since almost all DN  $\alpha/\beta$  T cells were Va24<sup>+</sup>.

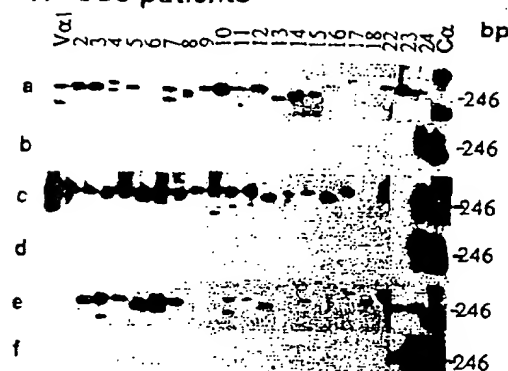
<sup>‡</sup> $p < 0.001$ .

crease of DN  $\alpha/\beta$  T cells in SSc patients was calculated to be 3.6- to 4.3-fold compared with those in healthy individuals. Thus, we isolated SP and DN populations from PBL by FACS<sup>®</sup> (Fig. 1 B). The purity of the fractionated DN samples was confirmed by reverse transcription-PCR with CD4 or CD8 primers (Fig. 1 C), and the TCRV $\alpha$  repertoire was then analyzed. As shown in Fig. 2, almost all families of TCRV $\alpha$  expression were observed in SP T cell fractions. Although individual Va gene expression varied in each sample, no significant difference was observed between SSc patients and healthy individuals.

On the other hand, very restricted TCRV $\alpha$  expression was noted in the DN T cell population. The only Va repertoire detected was Va24, and in some cases Va24 and Va23. Other TCRV $\alpha$  expression was below the level of detection by DNA blot analysis even with longer exposures (Fig. 2). The results indicate that the Va24 TCR repertoire dominates in peripheral DN T cells of both patients and healthy individuals. The dominant expression of Va24 TCR was further examined by quantitative PCR. Va24 expression in patients showed a four- to fivefold increase compared with healthy donors (Fig. 3). Together with the FACS<sup>®</sup> data, the results indicate that the number of DN T cells, particularly Va24-bearing T cells, is higher in patients, although the TCR- $\alpha$  repertoire in the DN population is restricted to Va24 in both SSc patients and healthy individuals.

**Oligoclonal Expansion of Va24 TCR in SSc Patients.** Porcelli et al. (6) and Dellabona et al. (7, 8) have shown that the Va24 TCR preferentially used in DN  $\alpha/\beta$  T cells in healthy donors is an invariant TCR encoded by Va24 and JaQ gene segments. Therefore, we attempted to compare Va24 TCR sequences in DN T cells between patients and

## A SSc patients



## B Healthy donors

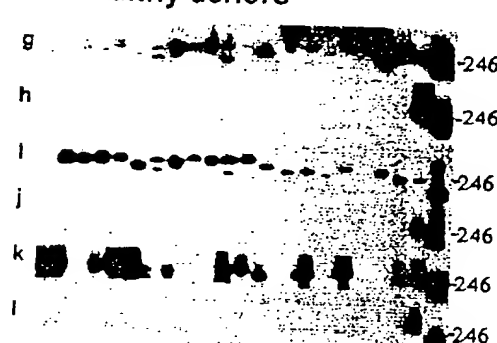


Figure 2. Analysis of TCRV $\alpha$  usage in SSc patients and healthy subjects. PCR products of the DN population (b, d, f, h, j, and l) and SP T cell fraction (a, c, e, g, i, and k) of three SSc patients (a-f) and three healthy subjects (g-l) were hybridized with the Ca probe. DNA size markers are shown to the right (in base pairs); Va markers are shown across the top.

healthy donors. The results are illustrated in Fig. 4. In healthy individuals, invariant Va24JaQ TCR was dominant at a high frequency (6/11, 6/7, and 5/11). However, among 13 in-frame Va24<sup>+</sup> cDNA clones in the patient SSc-1, four different Ja genes, IGRJa11, JaG, JaI, and JaAP511, were detected at a frequency of 5/13, 4/13, 3/13, and 1/13, respectively. Although several distinct Va24 TCR were expressed, we noted clonal expansion of invariant Va24 TCR other than Va24JaQ. Interestingly, the most dominant invariant Va24JaQ detected in healthy donors was not detected in patients. Similarly, in the patient SSc-2, 7 of 12 clones represented the Va24JaV TCR, three clones used the JaU gene, and two used the JaT gene. Again, we detected dominant expansion of invariant Va24JaV TCR different from Va24JaQ. In the patient SSc-3, seven different Ja genes (JaAA17, JaT, IGRJa11, IGRJa10, JaAF211, JaU, and JaAP511) were used at frequencies of 1/15 to 5/15. Among them, invariant Va24 JaAA17 dominated, while no Va24JaQ sequences were detected in the patient. Taken collectively, the Va24 rep-

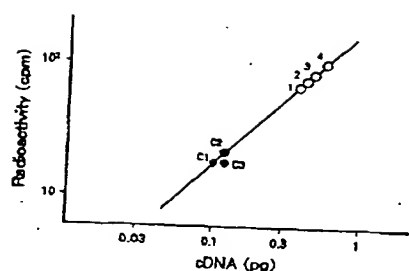


Figure 3. Quantitative PCR analysis of Va24<sup>+</sup> TCR. The number of DN T cells isolated was normalized by counting cell numbers. Total RNA was extracted and used for PCR to measure the frequency of Va24<sup>+</sup> TCR. PCR products were hybridized with <sup>32</sup>P-labeled Ca probe, and the intensities of autographic bands were quantitated by a densitometer. The radioactivity of varying concentrations (corresponding to 0.01–10 pg DNA) of the standard Va24 cDNA amplified by PCR was compared with that of PCR products from patients and healthy donors: 7, SSc-1; 2, SSc-2; 3, SSc-3; and 4, SSc-4 for SSc patients, and C1, C2, and C3 for healthy donors. The relative radioactivities of samples from four SSc patients are 67.9, 79.5, 89.5, and 93.1, respectively, all of which are significantly higher than the mean value of the control group (19.2 ± 1.2; *P* < 0.0005).

ertoire of DN  $\alpha/\beta$  T cells in PBL was basically heterogeneous, but with apparent oligoclonal expansion of invariant Va24 TCR in both patients and healthy individuals. However, in healthy donors, invariant Va24JaQ TCR always dominated, whereas, in SSc patients, invariant Va24JaQ TCR disappeared and oligoclonal expansion of other invariant Va24 TCR was observed.

**Selective Loss of T Cells Bearing Invariant Va24JaQ TCR.** As shown in Fig. 4, the invariant Va24JaQ TCR was found in healthy subjects at a high frequency (45–86% of total Va24 TCR), while it was not detected in SSc patients. To confirm the above findings, cDNA libraries generated by PCR were hybridized with the Va24 probe and the JaQ probe. Frequencies of invariant Va24JaQ TCR among total Va24 TCR sequences were estimated by the number of positive plaques and expressed as the ratio of invariant Va24JaQ to total Va24. As shown in Table 1, the invariant Va24JaQ TCR was hardly detected in all SSc patients (0.4%, 0%, 0%), while it was expressed at a high frequency of 21–50% in healthy donors. Based on the calculation of actual cell number of DN  $\alpha/\beta$  T cells, the number of T cells bearing invariant Va24JaQ was in the

Materials	Va 94	N	Ja 104	Frequency
<b>SScDN-1</b>				
GTGGTGAAC	GCAGAGGGCC	ATGGGAACAACAGACTCGCT-10RJ011		5/13
GTG	CGCACGAAGGGCTCTAGCAAAACAA	GCAAACTAATC-J00		4/13
GTGGTGAGC	CCTCGA	AACACCGACAAGCTCATC-J0X		3/13
GTGGTG	AGCGGTG	CAGGAACCTACAAATAGATC-J0AP511		1/13
<b>SScDN-2</b>				
GTGGTG	CCC00ACTCGAT	TCTGGGCTGGGAGTTACCAACTTACT-J0V		7/12
GTGGTG	GTATCACA	CGGTAAACAGTCTAT-J0U		3/12
GTGGTGAGC	GGA	GGAAGCTACATACCTACA-J0T		2/12
<b>SScDN-3</b>				
GTGGTG	ACCC0AAATA	CCG0CACTGCCAGTAACTACCC-J0AA17		5/15
GTGGTGAGC	CCTCCA	TCATCAGGAGGAAGCTACATACCTACA-J0T		2/15
GTGGTGAGC	TTCT	ATGGGAACAACAGACTCGCT-10RJ011		2/15
GTG	CTCT	CAATGACATGCGC-10RJ010		2/15
GTGGTG	AGTCCGAGA	ACTATGGTCAGAAATTTTGTG-J0AP211		2/15
GTGGTGAGC	GC0CTCT	CGGTAAACAGTCTAT-J0U		1/15
GTGGTG	TATA	CCTCAGGAACCTACAAATACATC-J0AP511		1/15
<b>Control-1</b>				
GTGGTGAGC		GACAGAGGCTCAACCTGGGG-J0Q		6/11
GTGGTGAGC	TCCACCTCT	TCAGGAACCTACAAATACATC-J0AP511		1/11
GTGGTGAGC	GAGAC	G0CAGGAAGTGTCTGATC-J0J		1/11
GTGGTGAGC	CCGTCCG	ACAATGCCAGACTCATG-J0R		1/11
GTG	TGGTGTCCG	ATTCAGGATACAGCACCTCACC-J0AD17		1/11
GTG	AGTGA	ATCAGGAGGAAGCTACATACCTACA-J0AP510		1/11
<b>Control-2</b>				
GTGGTGAGC		GACAGAGGCTCAACCTGGGG-J0Q		6/7
GTG	AGGGAT	TCAGGAACACACCTCTGTGTC-J0F		1/7
<b>Control-3</b>				
GTGGTGAGC		GACAGAGGCTCAACCTGGGG-J0Q		5/11
GTGGTGAGC	GCCTGT	TGGATAGCACTAT-J0B		1/11
GTGGTGAGC	GC0GGGTCCGGAG	AGGC-J0RJ0020		1/11
GTGGTGAGC	GAAAG	GGGAGGAGGAAACAACTACCC-J0JA2100		1/11
GTGGTG	ACCGATA	CCGGCACTGCCAGTAACTACCC-J0AA17		1/11
GTGGTGAGC	CGG	AACAATGACATGCGC-10RJ010		1/11
GTGGTG	GCCC	CATCAGGAGGAAGCTACATACCTACA-J0AP510		1/11

Figure 4. Junctional sequences of Va24 TCR obtained from DN  $\alpha/\beta$  T cells in SSc patients. The Va24<sup>+</sup> cDNA clones were randomly isolated from the PCR-amplified libraries from the DN population. Multiple isolates from each cDNA were sequenced. Nucleotide sequences of the 3' of TCR Va, N region, and the 5' of the Ja region are aligned. The frequency of identical sequences defined is shown in the right margin. These TCR Va and Ja sequences have been previously published (6, 20–23).

range of 0–0.4/mm<sup>3</sup> in SSc patients and 6.9–12.5/mm<sup>3</sup> in the healthy donors (see Table 1). This indicates that the decrease in the expression of invariant V $\alpha$ 24J $\alpha$ Q TCR is due to the selective loss of T cells bearing invariant V $\alpha$ 24J $\alpha$ Q, and not to the relative increase in the number of V $\alpha$ 24<sup>+</sup> T cells in SSc patients.

The predominant expression of invariant V $\alpha$ 14J $\alpha$ 281 TCR on the DN T cell population in the periphery has been reported in mice (18, 19). The most characteristic feature of invariant V $\alpha$ 14 TCR with regard to autoimmune diseases is that there is a striking inverse correlation between autoimmune disease development and the expression of invariant V $\alpha$ 14 TCR. In fact, invariant V $\alpha$ 14 expression declines selectively with time after birth and disappears when mice develop autoimmune diseases in (NZB  $\times$  NZW)F<sub>1</sub>, *lpr*, or *gld* mice (9). Moreover, the treatment of young *lpr* mice with anti-V $\alpha$ 14 antibody *in vivo* induces splenomegaly at least threefold greater than that in untreated *lpr* mice at the same age, indicating the augmentation and acceleration of lymphoproliferative disorders. These results suggest that invariant V $\alpha$ 14 T cells function as regulatory T cells that control autoimmune disease development. Based on the above analogy to murine invariant V $\alpha$ 14 TCR, it is likely that human invariant

V $\alpha$ 24J $\alpha$ Q TCR should play a decisive role in regulating the development of autoimmune disease.

Contrary to the invariant V $\alpha$ 24J $\alpha$ Q TCR, DN  $\alpha/\beta$  T cells in SSc patients showed oligoclonal expansion of invariant V $\alpha$ 24 TCR other than V $\alpha$ 24J $\alpha$ Q, including V $\alpha$ 24 IGRJ $\alpha$ 11, V $\alpha$ 24J $\alpha$ G, V $\alpha$ 24J $\alpha$ V, and V $\alpha$ 24J $\alpha$ AA17 (Fig. 4). Our previous studies have shown that the TCRV $\beta$  repertoire in DN T cells is limited to one or two V $\beta$  genes in an individual patient, such as V $\beta$ 5, V $\beta$ 7, or V $\beta$ 11 (15). Because invariant V $\alpha$ 24J $\alpha$ Q TCR has been shown to be associated with skewed V $\beta$ , such as V $\beta$ 2, V $\beta$ 8, V $\beta$ 11, and V $\beta$ 13 (6–8, 10), the restricted V $\beta$  usage that predominated in patients was different from that seen in healthy donors. In addition, these invariant V $\alpha$ 24 TCR sequences were not detected in healthy individuals. Thus, they might be unique to SSc patients, suggesting that the expanded oligoclonal invariant V $\alpha$ 24 TCR in SSc patients could be autoaggressive T cells in autoimmune status. Sequence differences in the oligoclonal V $\alpha$ 24 TCR that dominate in SSc patients might reflect differences in the polymorphism of restriction elements or in epitope specificities. The establishment of T cells bearing invariant V $\alpha$ 24 TCR other than V $\alpha$ 24J $\alpha$ Q from SSc patients will provide a clue to the mechanisms of autoimmune diseases.

We thank Dr. Margaret Dooley Ohto for her critical reading of the manuscript and Ms. Hiroko Tanabe for editing and preparation of the manuscript.

This work was supported in part by grants from the Japanese Ministry of Health and Welfare and the Japanese Ministry of Science and Culture.

Address correspondence to Dr. Takayuki Sumida, Division of Rheumatology and Molecular Immunology, Institute of Medical Science, St. Marianna University, School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki, Japan 216.

Received for publication 10 April 1995 and in revised form 14 June 1995.

## References

1. Fowlkes, B.J., A.M. Kruisbeek, H. Ton-That, M.A. Weston, J.E. Coligan, R.H. Schwartz, and D.M. Pardoll. 1987. A novel population of T-cell receptor  $\alpha\beta$ -bearing thymocytes which predominantly express a single V $\beta$  gene family. *Nature (Lond.)* 329:251–254.
2. Takahama, Y., S.O. Sharrow, and A. Singer. 1991. Expression of unusual T cell receptor (TCR)-V $\beta$  repertoire by Ly-6C<sup>+</sup> subpopulations of CD4<sup>+</sup> and/or CD8<sup>+</sup> thymocytes: evidence for a developmental relationship between Ly-6C<sup>+</sup> thymocytes and CD4<sup>+</sup>8<sup>+</sup> TCR- $\alpha\beta$ <sup>+</sup> thymocytes. *J. Immunol.* 147:2883–2891.
3. Bix, M., M. Coles, and D. Raulet. 1993. Positive selection of V $\beta$ 8<sup>+</sup>CD4<sup>+</sup>8<sup>+</sup> thymocytes by class I molecules expressed by hematopoietic cells. *J. Exp. Med.* 178:901–908.
4. Makino, Y., R. Kanno, T. Ito, K. Higashino, and M. Taniguchi. 1995. Predominant expression of invariant V $\alpha$ 14<sup>+</sup> TCR  $\alpha$  chain in NK1.1<sup>+</sup> T cell populations. *Int. Immunol.* 7: 1157–1161.
5. Lantz, O., and A. Bendelac. 1994. An invariant T cell receptor  $\alpha$  chain is used by a unique subset of major histocompatibility complex class I-specific CD4<sup>+</sup> and CD4<sup>+</sup>8<sup>+</sup> T cells in mice and humans. *J. Exp. Med.* 180:1097–1106.
6. Porcelli, S., C.E. Yockey, M.B. Brenner, and S.P. Balk. 1993. Analysis of T cell antigen receptor (TCR) expression by human peripheral blood CD4<sup>+</sup>8<sup>+</sup>  $\alpha/\beta$  T cells demonstrates preferential use of several V $\beta$  genes and an invariant TCR  $\alpha$  chain. *J. Exp. Med.* 178:1–16.
7. Dellabona, P., G. Casorati, B. Friedli, L. Angman, F. Sallusto, A. Tunnaccliff, E. Roosneek, and A. Lanzavecchia. 1993. In vivo persistence of expanded clones specific for bacterial antigens within the human T cell receptor  $\alpha/\beta$  CD4<sup>+</sup>8<sup>+</sup> subset. *J. Exp. Med.* 177:1763–1771.
8. Dellabona, P., E. Padovan, G. Casorati, M. Brockhaus, and A. Lanzavecchia. 1994. An invariant V $\alpha$ 24-J $\alpha$ Q/V $\beta$ 11 T cell receptor is expressed in all individuals by clonally expanded CD4<sup>+</sup>8<sup>+</sup> T cells. *J. Exp. Med.* 180:1171–1176.

9. Makino, Y., H. Koseki, Y. Adachi, T. Akasaka, K.-I. Tsuchida, and M. Taniguchi. 1994. Extrathymic differentiation of a T cell bearing invariant V $\alpha$ 14J $\alpha$ 281 TCR. *Int. Rev. Immunol.* 11:31-46.
10. Brooks, E.G., S.P. Balk, K. Aupex, M. Colonna, J.L. Strominger, and V. Groh-Spies. 1993. Human T-cell receptor (TCR)  $\alpha/\beta^+$  CD4 $^-$ CD8 $^-$  T cells express oligoclonal TCRs, share junctional motifs across TCR V $\beta$ -gene families, and phenotypically resemble memory T cells. *Proc. Natl. Acad. Sci. USA.* 90:11787-11791.
11. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. 1980. Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis & Rheum.* 23: 581-590.
12. Sumida, T., F. Yonaha, T. Maeda, E. Tanabe, T. Koike, H. Tomioka, and S. Yoshida. 1992. T cell receptor repertoire of infiltrating T cells in lips of Sjögren's syndrome patients. *J. Clin. Invest.* 89:681-685.
13. Sumida, T., Y. Kita, F. Yonaha, T. Maeda, I. Iwamoto, and S. Yoshida. 1994. T cell receptor V $\alpha$  repertoire of infiltrating T cells in labial salivary glands from patients with Sjögren's syndrome. *J. Rheumatol.* 21:1655-1661.
14. Sottini, A., L. Imberti, R. Gorla, R. Cattaneo, and D. Primi. 1991. Restricted expression of T cell receptor V $\beta$  but not V $\alpha$  genes in rheumatoid arthritis. *Eur. J. Immunol.* 21:461-466.
15. Sakamoto, A., T. Sumida, T. Maeda, M. Itoh, T. Asai, H. Takahashi, S. Yoshida, T. Koike, H. Tomioka, and S. Yoshida. 1992. T cell receptor V $\beta$  repertoire of double-negative  $\alpha/\beta$  T cells in patients with systemic sclerosis. *Arthritis Rheum.* 35:944-948.
16. Maddon, P.J., D.R. Littman, M. Godfrey, D.E. Maddon, L. Chess, and R. Axel. 1985. The isolation and nucleotide sequence of a cDNA encoding the T cell surface protein T4: a new member of the immunoglobulin gene family. *Cell.* 42: 93-104.
17. Littman, D.R., Y. Thomas, P.J. Maddon, L. Chess, and R. Axel. 1985. The isolation and sequence of the gene encoding T8: a molecule defining functional classes of T lymphocytes. *Cell.* 40:237-246.
18. Koseki, H., K. Imai, F. Nakayama, T. Sado, K. Moriwaki, and M. Taniguchi. 1990. Homogenous junctional sequence of the V14 $^+$  T-cell antigen receptor  $\alpha$  chain expanded in unprimed mice. *Proc. Natl. Acad. Sci. USA.* 87:5248-5252.
19. Makino, Y., N. Yamagata, T. Sasho, Y. Adachi, R. Kanno, H. Koseki, M. Kanno, and M. Taniguchi. 1993. Extrathymic development of V $\alpha$ 14-positive T cells. *J. Exp. Med.* 177: 1399-1408.
20. Roman-Roman, S., L. Ferradini, J. Azocar, C. Genevée, T. Hercend, and F. Triebel. 1991. Studies on the human T cell receptor  $\alpha/\beta$  variable region genes I. Identification of 7 additional V $\alpha$  subfamilies and 14 J $\alpha$  gene segments. *Eur. J. Immunol.* 21:927-933.
21. Yoshikai, Y., N. Kimura, B. Toyonaga, and T.W. Mak. 1986. Sequences and repertoire of human T cell receptor  $\alpha$  chain variable region genes in mature T lymphocytes. *J. Exp. Med.* 164:90-103.
22. Klein, M.H., P. Concannon, M. Everett, L.D. Kim, T. Hunkapiller, and L.E. Hood. 1987. Diversity and structure of human T-cell receptor  $\alpha$ -chain variable region genes. *Proc. Natl. Acad. Sci. USA.* 84:6884-6888.
23. Kimura, N., B. Toyonaga, Y. Yoshikai, R. Du, and T.W. Mak. 1987. Sequences and repertoire of the human T-cell receptor  $\alpha$  and  $\beta$  chain variable region genes in thymocytes. *Eur. J. Immunol.* 17:375-383.